

# Oil-Red-O staining to determine the amount of stored triglyceride in *Caenorhabditis elegans*

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Vitellogenin-2 acts downstream of PRY-1/Axin to regulate lipids and lifespan in *C. elegans*

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## Detailed protocol

1. Oil-Red-O staining was conducted by washing 200-300 synchronized day-1 adults with 1x PBS. Synchronized animals were obtained by bleach treatment.
2. Worms were then washed three times with 1x PBS pH 7.4 and allowed to settle by gravity.
3. To permeabilize the cuticle, worms were resuspended in 60 µl of PBS to which 120µL of 2x MRWB buffer and 60 µl of 4% paraformaldehyde (PFA) was added (total volume 240 ml).  
2X MRWB was made using the protocol described earlier (Soukas et al., 2009).  
(160 mM KCl, 40 mM NaCl, 14 mM Na2EGTA, 1 mM spermidinHCl, 0.4 mM spermine, 30 mM Na-PIPES pH 7.4, 0.2% β-mercaptoethanol **add just before using**).
4. Samples were gently rocked for 1h at room temperature (allowing animals to rock inside the tube, without spreading the liquid over the whole tube). Animals were allowed to settle by gravity, and after aspirating the buffer they were subjected to 3 freeze-thaw in an alcohol bath (dry ice and ethanol).
5. Worms were washed with 1x PBS to remove PFA. Subsequently, they were resuspended in 60% isopropanol and allowed to dehydrate by incubating at room temperature for 15 minutes.
6. Oil-Red-O was prepared as follows: 0.5g/100mL stock solution (in isopropanol) was equilibrated for several days. The solution was freshly diluted to 60% with water and rocked for at least 1h. [filtered with 0.45 or 0.22µm-filter.]
7. Worms (from step 5) were allowed to settle. Isopropanol was carefully removed. 1 mL of 60% filtered Oil-Red-O stain was added, and animals were incubated for at least 48 hours with rocking.
8. Dye was removed and 200 µL of 1x PBS 0.01% Triton X-100 was added to worms.
9. Stained worms were mounted and imaged using a Leica microscope outfitted with DIC optics following the previously described protocol (O'Rourke et al., 2009).

### References:

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- Soukas, A.A., Kane, E.A., Carr, C.E., Melo, J.A., and Ruvkun, G. (2009). Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in *Caenorhabditis elegans*. *Genes Dev.* 496–511.

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Mallick, A. and Gupta, B. (2021). Oil-Red-O staining to determine the amount of stored triglyceride in *Caenorhabditis elegans*. Bio-protocol Preprint. [bio-protocol.org/prep934](https://bio-protocol.org/prep934).
2. Mallick, A. and Gupta, B. P. (2020). Vitellogenin-2 acts downstream of PRY-1/Axin to regulate lipids and lifespan in *C. elegans*. microPublication Biology. DOI: [10.17912/micropub.biology.000281](https://doi.org/10.17912/micropub.biology.000281)

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